

Comparative Studies between the Effects of Antibiotic (Oxytetracycline); Probiotic and Acidifier on E. coli Infection and Immune Response in Broiler Chickens

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Abstract: In this experiment, the efficacy of antibiotic (oxytetracycline); Nutrilac® as acidifier, lactiflora® plus as probiotics mixtures) were compared against E coli O78 infection and immune response to routine vaccination (Newcastle disease (ND) and Infectious Bronchitis virus (IB) and inactivated avian influenza (AI) vaccine) in broiler chickens. A total of 250, 1 Day- old Arbor Acers Broiler chicks were divided into 5 equal groups(1-5) 50 chicks per each) group 1 were kept as blank control. Chicks of group 2 were infected orally with 0.5ml of E coli O78 containing 1×10^4 viable organism /ml in phosphate buffered saline (PBS) and kept as infected control. Chicks of group 3 were received Nutrilac® in water (3ml/liter). Chicks of group 4 were received lactiflora® in feed (1 g/kg.). Chicks of group 5 were received Oxytetracyclin 20% in feed (1g/kg.) At 12 days of age chickens of groups 3- 5 were orally inoculated with 0.4 ml of phosphate buffered saline (PBS) containing 1×10^4 viable organism /ml of E coli (O78) by the same dose of group 2. Our results showed the, mortality was highest in groups infected with E.coli (17 bird) followed by those receive, lactiflora® plus and oxytetracyclin (6 bird/ group) then the lowest were both negative control and Nutrilac (5 bird) while weight gain in all chicken groups the Highest weight gain was those of group receiving Nutrilac® (605) followed by group receive lactiflora® plus(600) then group receive oxytetracyclin(583) and negative control (541). lowest weight gain was those receive E.coli. The immune response to routine vaccination against live Newcastle disease virus (NDV) vaccine; Infectious Bronchitis virus (IB) vaccine and inactivated Avian Influenza vaccine (AI) in the same chicken groups was revealed highest titer with Lactiflore plus followed by Nutrilac then oxytetracyclin then blank control. lowest immune response was showed in infected control group. Histopathological examination for second group revealed that liver central and portal veins were moderately to markedly dilated and congested in almost all samples. Changes in the hepatic parenchyma varied from diffuse and marked vascular degeneration in which the nuclei were either pyknotic or karyolysis. Hepatic necrosis which occurs either in the form of minutes sporadic necrotic foci, or variable sized multifocal areas of necrosis infiltrated with mononuclear cells were seen also. In some cases large area of hepatic necrosis were seen. The hepatocytes in the necrotic area either disappeared or showed pyknotic nuclei and or showed large vesicular nuclei with peripheral chromatin. Intestine showing diffused degeneration of the mucosa and desquamation of the epithelial cells that accumulate in the lumen with hyalinization, some field showing necrotic and desquamated epithelials, and heavily mononuclear cells infiltrated L.propria and congested submucosa. In conclusion It could be concluded that probiotic and acidifier has great value on poultry production as it act as growth promoter either by enhancing digestibility or competitive inhibition of colonization of pathogenic bacteria which destruct intestinal wall and produce toxins. Also results were showed those probiotics and acidifier are of positive value in immune response for vaccination.

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1. Introduction

The association of E. coli with certain pathological conditions of poultry dates from the end of the last century. Many reports on this subject have been published (*Hjarr'e & Wramby, 1945; Sojka & Carnagham, 1961; Sojka, 1965; Emery et al., 1992; Barnes & Lozano, 1994; Morris, 1994; Dhillon & Jack, 1996*). Generally, E. coli affects poultry of all ages, although young birds are more sensitive. The infection is considered to be one of the leading causes of economic loss in the poultry industry.

Different experimental E. coli infection have been described: septicemia, enteritis, granulomas,

omphalitis, sinusitis, airsacculitis, arthritis/synovitis, peritonitis, pericarditis, cellulitis, swollen head syndrome, etc. More frequently, E. coli disease occurs as a consequence of the adverse influence of factors such as ammonia, moisture, dust, hormones or infectious agents such as viruses and mycoplasmas (*Oyetunde et al., 1978; Weinach et al., 1984; Gross, 1990; Leitner and Heller, 1992*).

Sometimes E. coli is the primary cause of disease, particularly in young birds (*Cheville and Arp, 1978*) and in adults (*Dhillon and Jack, 1996*). Many serotypes of E. coli have been isolated throughout the world (*Sojka and Carnagham, 1961; Glanz et al.,*

1962; Rosenberger and Chand, 1981 and Cloud et al., 1985). Currently, 173 O, 74 K, 53 H and 17 F antigens are recognized (Barnes and Gross, 1997), but the most frequently occurring serotypes in poultry are O1, O2, O8, O35 and O78. Pathological conditions due to *E. coli* have been reproduced experimentally by different routes of inoculation, with mortality varying from 30 to 100% (Gross, 1957 and Sojka and Carnaghan, 1961).

In modern broiler management, preventive measures are taken to control of such diseases and bacterial enteritis, which reduce feed utilization and live performance characteristics. Probiotic feed additives are frequently used for this purpose Zohair (2006).

There are public concerns that the use of antibiotic feed additives in animals may give a rise to the bacterial resistance to human therapeutic drugs, especially those antibiotics that are closely related to human drugs (Witte, 1997 and Aarestrup et al., 1998). Moreover, there are some concerns on the effect of feed additives on the gut flora composition, specifically in regard to increased excretion of food-borne pathogens (Williams Smith and Tucker, 1975, 1980). These arguments put pressure on the future of feeding low-level antibiotics to animal

Probiotic which are live microbial feed supplements that beneficially affect the host animal by altering its intestinal microbial balance (Nurmi and Rantala, 1973), have been consumed for centuries, either as natural components of food, or as fermented foods. Numerous in vivo and in vitro studies since then have shown that the commensal intestinal microbiota inhibit pathogens, that disturbances of the intestinal microbiota can increase susceptibility to infection, and that addition of probiotics increase resistance to infection (Rolfe, 2000). The same finding with Guo et al. (2006) confirm that some isolates of probiotics have inhibitory activity against strains of *E. coli* K88 and K99 that was explained by Zohair (2006) who found that probiotics in broiler chickens ration has capability to reduce colonization of *E. coli* in intestine together with reducing both mortalities, severity of postmortem and histopathological lesions. On the other hand Mountzouris et al. (2007) investigated the efficacy of some probiotic strains and reported that it display growth promoting effect that did not differ from antibiotic used as growth promoter. Talebi et al. (2008) stated that not only the use of probiotics significantly enhanced broiler performance by improving body weight and decreasing feed conversion ratio but also improve the antibody responses to Newcastle disease virus and infectious bursal disease vaccination but the antibody titers of the probiotic treated group were not significantly different from those not receiving probiotics.

This experiment aimed to compare the effect of probiotic, acidifier with the effect of antibiotic on the

on gastrointestinal tract (GIT) integrity, bird performance after challenge with *E. coli* O78 with keeping eye other effect on antibody response for some important viral infection vaccines in Arbor Acers broilers under commercial conditions

2. Material and Methods

Chicks

Day old 250 commercial Arbor Acers Broiler chicks were used in the study.

The used birds were divided into 5 equal groups of 50 birds of each. Chicks of group 1 were (negative control group). Chicks of group 2 were infected orally with 0.5ml of *E. coli* O78 K80 H11 strains containing 1×10^4 viable microorganism /ml phosphate buffered saline (PBS) and received feed and water without any additive (positive control group). Chicks of group 3 were received Nutrilac in water (3ml/liter). Chicks of group 4 were received lactiflore plus in feed (1 g/kg.). Chicks from group 5 were received Oxytetracycline 20% in feed (1g/kg.). Chicks were floor reared in separate rooms and kept in environmentally controlled rooms. Chicks of all groups were vaccinated via drinking water against Newcastle disease and infectious bronchitis using Hitchner B1+ IB_{H120} vaccines at 7th day of age and against Avian influenza vaccine was given subcutaneously at 10th day of age 0.5 MI of H5N2 vaccine. Revaccination against ND using LaSota vaccine and vaccination against IBD using Bursine plus^R IBDV vaccine were given at 14th day of age. . At 12 d of age chickens from groups 2 to 5 (50 birds / each) were orally inoculated with 0.5 ml of PBS containing 1×10^4 /ml viable organism of *E. coli* (O78) according to Sarhan (1977)

Viral vaccines

1. HB1 vaccine: forteDodge, batch no.1084283A, 1000 dose.
2. Lasota vaccine: Scherring, batch no. 94020030, 1000 dose.
3. H120 vaccine : Intervet, batch no. 11623LJ01.
4. Influnza vaccine: Borhinger, batch no. 1107127A.

Bacterial strains

E. coli strain [O78 K80 H11] used At 12 days of age for groups 2 to 5 were orally inoculated with 0.5 phosphate buffered saline (PBS) containing 1×10^4 viable organism /ml according to Sarhan (1977)

Probiotics (lactiflora[®] plus)

Contain enterococcus faecium 4×10^7 C.F.U; *Pedococcus acidolactic* 2×10^7 C.F.U; and Calcium as carbonate up to 1.0 gram. It was used in feed in rate of 1 g/kg

Acidifire (Neutrilac[®])

Contained formic acid and lactic acid it was used in rate of 3ml/liter via drinking water.

Antibiotic used

Oxytetracycline 20% in feed in a dose of 1g/kg feed.

Serological examination

Blood samples were collected from 20 birds from each group (total 100 samples from all groups) at the end of the experiment (42 day old). Serum samples were tested to evaluate the antibodies titer against Newcastle disease and Avian influenza, using the standard HI method. The test was carried out according to the standard procedure described by *Majiyagbe and Hitchner (1977)* the end point were estimated according to scheme described by *Kaleta and Siegmann (1971)*. While serological examination of Infectious Bronchitis, carried out using commercial Elisa system (IDEXX Coporation, Wetbrook, USA) according to manufacturers instructions.

Histopathological assay

Samples were collected and preserved in 10% natural formalin. The specimens were processed stained by Hematoxylin-Eosine (H&E) stain which, was prepared according to *Culling (1973)* and examined microscopically for any evidence of histopathological changes.

3. Result and Discussion

As summarized in table (1), mortality was highest in groups infected with E.coli (17 bird) followed by those receive Lactiflor plus and oxytetracyclin are the same (6 bird) then the lowest were both negative control and Nutrilac (5 bird) these result confirm that E.coli play an important role in disease occur in poultry especially when it become complicating factor also colibacillosis in poultry causing outbreaks especially when occurring secondarily when host defenses have been impaired or overwhelmed by virulent E.coli strains *Barnes(2000)*.

In table (2) weight gain in all chicken groups by the end of experiment the highest weight gain was those of group receiving **Neutrilac®** (605) followed by group receive Lactiflore plus(600) then group receive oxytetracyclin(583) then negative control (541) and the lowest was those receive E.coli. this could be explained results in second group, while E.coli colonized in intestinal wall and affect intestinal integrity that reflected on feed gain while in groups receiving probiotics(group 3) and acidifier (group 4) were the highest which was explained by *Jin et al.(1997)* who summarized probiotic effect in four points 1st as it maintain normal intestinal microflora by competitive exclusion and antagonism second by ultrating metabolism by increasing digestive enzyme

activity and decreasing bacterial activity and ammonia production third by neutralizing enterotoxins forth by stimulating the immune system those was matched also with result of *Awaad et al.(2009)* who found that adding probiotic in broiler ration resulting in improving performance ,carcass yield , organs weights which was matched also with result of *Mountzouris et al.(2010)* and *Taheri et al.(2010)* parallel result obtained by *Zohair(2006)* on capability of organic acid in reduce colonization of pathogenic microorganism and improve digestability and weight gain.the result also proved that probiotics and acidifier has the same beneficial effect on weight gain as those of antibiotics these was committed with the result of *Mountzouris et al.(2007)* and *Ashayerizadeh et al(2009)*. In conclusion on that table we should take in consideration that any game increase in broiler body weight is of great value commercially.

Serological test for each groups to evaluate antibody response for Newcastle disease virus(NDV), Avian Influenza and Infectious Bronchitis virus (table 3 and table 4) revealed that the highest titer was with Lactiflore plus followed by Nutrilac then oxytetracyclin then negative control while the lowest was positive control infected with E.coli this result was matched with *Yasui and Ohawki(1991)* who found that probiotics organisms support the immunostimulatory properties and interact with the immune system at many levels also *Kabir et al.(2004)* reported significant higher antibody production (P<0.01) in experimental broilers as compared to control one , which also assisted by *Koenen et al.(2004)* that those probiotics and acidifier have appositive effect on humoral and cellular immune responses in layer and meat type chickens species as well as results obtained by *Haghighi et al.(2006)* and *Ogawa et al.(2006)* on the other hand *Talebi et al(2008)* found that inspite of probiotic improve the antibody responses to newcastle disease virus and infectious bursal disease vaccination but the antibody titers of the probiotic treated group were not significantly different from those not receiving probiotic which was matched with our result, finally *Sohail et al.(2010)* stated that use of dietary supplementations improve humoral immunity against both NDV and IBD virus.

Concerning the histopathological alteration in positive control group infected with E.coli (group two) and to examine its effect simulating the field conditions and explain the great negative effect on performance compared with other four groups found that liver central and portal veins were moderately to markedly dilated and congested in almost all samples. changes in the hepatic parenchyma varied from diffuse and marked vascular degeneration (fig 1) in which the nuclei were either pyknotic or karyolysis fig(2). Hepatic necrosis which occurs either in the form of minutes sporadic necrotic foci (fig 3), or variable sized

multifocal areas of necrosis infiltrated with mononuclear cells could be seen (fig 4) . in some cases large area of hepatic necrosis were seen (fig 5). The hepatocytes in the necrotic area either disappeared or showed pyknotic nuclei and or showed large vesicular nuclei with peripheral chromatine (fig 6).intestine showing diffused degeneration mucosa (fig 7) and desquamated epithelial cells that accumulate in the lumen with hyalinization (fig 8) ,some field showing necrosed and descumated l.epithelials ,and heavily mononuclear cells infiltrated l.propria and congested submucosa (fig 9) this finding this was matched with result found by *Eysen(1971) and Coates(1971)* when found that intestinal reaction against several types of

microorganisms in the form of thickening of the intestinal wall and loss of microstructure . also *Saif(2008)* concluded that infection of E.coli has various histopathological effect on different organs such spleen, kidney and liver and he reported that E coli cause acute necrosis of hepatocytes.

It could be concluded that probiotic and acidifier of great value on modern poultry production as it act as growth promoter either by inhancing digestibility or competitive inhibition of colonization of pathogenic bacteria which distruct intestinal wall and produce toxins . also those probiotics and acidifier are of positive value in immune response for vaccination.

Table 1. Mortality percentage/ week of the chicken groups:

Group	1 st Week		2 nd Week		3 rd Week		4 th Week		5 th Week		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
Negative * control	1	2.0	1	2.0	2	4.1	1	2.1	0	0.0	5	10.0
Positive control	2	4	6	12.5	5	11.9	2	5.1	2	5.4	17	34.0
Lactiflore plus	0	0.0	3	6.0	2	4.2	0	0.0	1	2.2	6	12.0
Nutrilac	0	0.0	2	4.0	2	4.16	1	2.17	0	0.0	5	10.0
Oxytetracyclin	1	2.0	1	2.0	2	4.1	1	2.1	0	0.0	5	12.0

No= number of birds

Table (2). Weight gain in grams in all chickens groups:

Group	1 st Week		2 nd Week		3 rd Week		4 th Week		5 th Week	
	Weight /gm	Weight gain/gm	Weight/g m	Weight gain/gm	Weigh t/gm	Weight gain/gm	Weight/ gm	Weight gain/gm	Weight/ gm	Weight gain
Negative control	175	131	418	243	837	419	1398	561	1939	541
Positive control	173	129	398	225	808	410	1289	481	1789	500
Lactiflore plus	172	128	421	249	848	427	1410	562	2010	600
Nutrilac	169	125	412	243	857	445	1345	488	1950	605
Oxytetra cyclin	174	130	436	262	877	441	1518	641	2102	583

Table (3): mean antibody titer against NDV and avian influenza using HI test

	ND HI	AI HI
Negative control	3.8	1.98
Positive control	2.3	1.8
Lactiflore plus	4.6	2.2
Nutrilac	4.1	2.3
Oxytetracyclin	3.7	2.03

Table (4) mean antibody titer against infectious bronchitis virus using ELISA test:

Groups	IB Elisa
Negative control	879
Positive control	751
Lactiflore plus	1002
Nutrilac	987
Oxytetracyclin	978

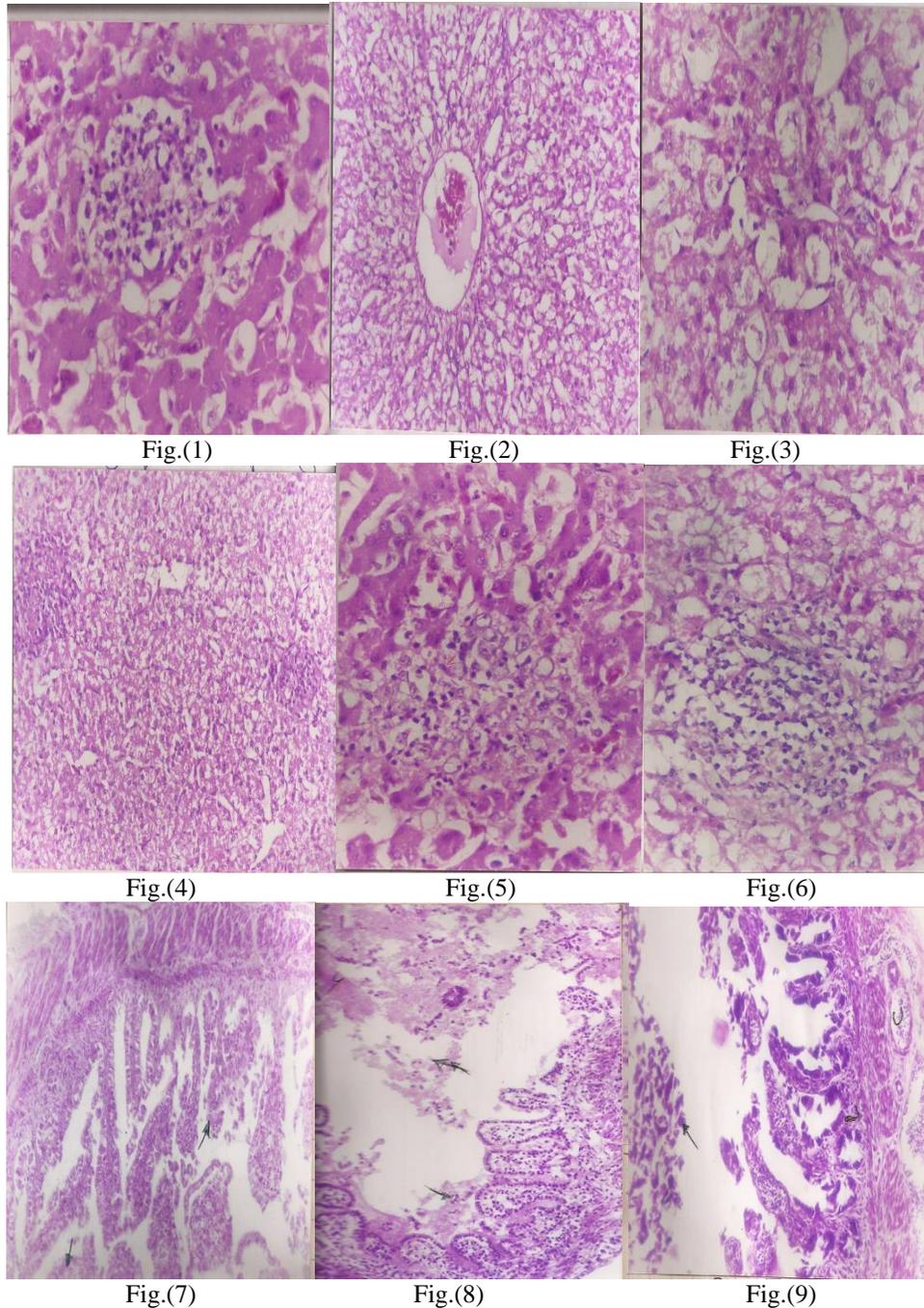


Fig.1 liver showing minute sporadic necrotic foci together with sinusoidal dilatation and pressure atrophy of some hepatocytes (H&E x 400)

Fig. 2 liver showing marked and diffuse vascular degeneration of hepatocytes (H&Ex200)

Fig. 3 higher magnification showing pyknosis and karyosis of the cell nuclei (H&Ex400).

Fig. 4 liver showing diffuse vascular degeneration of hepatocytes with multifocal areas of necrosis infiltration with mononuclear cells (H&Ex100)

Fig. 5 liver showing area of hepatocellular necrosis with disappearance of some hepatocytes, other showed either pyknotic and or large vesicular nuclei with peripheral chromatin (arrows) (H&Ex400).

Fig. 6 liver showing area of hepatic necrosis(H&Ex400).

Fig. 7 intestine of chickens showing diffused degeneration mucosa (arrows) (H&EX200)

Fig. 8 intestine of chicken showing desquamated epithelial cells (arrows) that accumulate in the lumen with hyalinization (h)(H&EX200).

Fig. 9 intestine of chicken showing necrosed and descumated l.epithelials (arrows), heavily mononuclear cells infiltrated l.propria (L) and congested submucosa (C), (H&EX200)

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